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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/12, 15/62, C07K 14/715, 19/00, A61K 38/17</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/37772</b> <b>(43) International Publication Date:</b> 29 July 1999 (29.07.99)
<b>(21) International Application Number:</b> PCT/US99/01419 <b>(22) International Filing Date:</b> 22 January 1999 (22.01.99)  <b>(30) Priority Data:</b> 60/072,301 23 January 1998 (23.01.98) US 60/078,835 20 March 1998 (20.03.98) US 60/094,469 28 July 1998 (28.07.98) US  <b>(71) Applicant (for all designated States except US):</b> IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SIMS, John, E. [US/US]; 4207 43rd Avenue N.E., Seattle, WA 98105 (US). BORN, Teresa, L. [US/US]; 11328 38th Avenue N.E., Seattle, WA 98125 (US).  <b>(74) Agent:</b> HENRY, Janis, C.; Immunex Corporation, Law Dept., 51 University Street, Seattle, WA 98101 (US).		<b>(81) Designated States:</b> AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HR, HU, ID, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> IL-18 RECEPTORS  <b>(57) Abstract</b>  A polypeptide that functions as an IL-18 receptor is disclosed. The receptor is multimeric and includes at least one AcPL polypeptide, or fragment thereof, and at least one IL-1R $\alpha$ polypeptide, or fraction thereof. The receptor binds IL-18 and finds use in inhibiting biological activities mediated by IL-18.		

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## IL-18 RECEPTORS

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### BACKGROUND OF THE INVENTION

#### Field of the Invention

The present invention relates to proteins that are members of the IL-1 receptor family. More particularly, the present invention relates to IL-1Rrp1 and AcPL receptor complexes that mediate high affinity IL-18 binding and activity as well as inhibit IL-18 mediated activity.

#### Description of Related Art

The type I interleukin-1 receptor (IL-1RI) mediates the biological effects of interleukin-1, a pro-inflammatory cytokine (Sims et al., *Science* 241:585-589, 1988; Curtis et al., *Proc. Natl. Acad. Sci. USA* 86:3045-3049, 1989). A second interleukin-1 receptor (designated type II IL-1R or IL-1RII) binds IL-1, but does not appear to mediate signal transduction (McMahan et al., *EMBO J.* 10:2821, 1991; Sims et al., *Proc. Natl. Acad. Sci. USA* 90:6155-6159, 1993). IL-1RI and IL-1RII each bind IL-1 $\alpha$  and IL-1 $\beta$ . IL-1 has been implicated in chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease. There is increasing evidence that IL-1 plays a role in osteoporosis. All of these activities are initiated by the signaling function of the cytoplasmic portion of the Type I IL-1R. IL-1ra inhibits the activities of IL-1 by binding to the type I IL-1 receptor, thereby blocking access to IL-1 $\alpha$  and IL-1 $\beta$  while eliciting no biological response of its own.

IL-1RI and IL-1RII belong to a family of proteins that exhibit significant sequence homology. One such protein is IL-1R accessory protein (IL-1R AcP), described in Greenfeder et al. (*J. Biol. Chem.* 270: 13757-13765, 1995). This protein, by itself, is not capable of binding IL-1, but does form a complex with IL-1RI and IL-1 $\alpha$  and IL-1 $\beta$ . When co-expressed with IL-1RI, recombinant IL-1R AcP increases the binding affinity of IL-1RI for IL-1 $\beta$  (Greenfeder et al., *supra*).

Another protein exhibiting sequence homology to the IL-1RI and IL-1RII family is the IL-1 receptor related protein 1 (IL-1Rrp1) (See Parnet et al. *J. Biol. Chem.* 271:3967, 1996, and Torigoe et al., *J. Biol. Chem.* 272:25737, 1997). Still another such protein is AcPL.

IL-18 is a homologue of IL-1 $\alpha$  and IL-1 $\beta$  and is known to activate many of the same responses activated by IL-1. For example, cells stimulated with IL-18 activate

5 NF $\kappa$ B and produce known inflammatory mediators. IL-18 acts as a stimulator of Th1 cell growth and differentiation and is a potent inducer of  $\gamma$ -interferon production from Th1 cells. The Th1 class of helper T cells are known to mediate inflammatory reactions. IL-18 enhances NK cell killing activity and has been implicated in septic shock, liver destruction, inflammatory bowel disease and diabetes.

10 Recently it was shown that IL-1Rrp1 binds IL-18 and mediates IL-18 signaling in transfected cells. However, the IL-1Rrp1 binding affinity for IL-18 is very low and it is likely that one or more additional receptors or receptor subunits are involved with IL-18 binding and signaling.

15 Thus, the identification of additional receptors of for IL-18 is desirable. Such receptor proteins can be studied to determine whether or not they bind IL-18 and, if so, whether the receptors play a role in mediating signal transduction. Furthermore, soluble forms of such receptors may be used to inhibit IL-18 activity and ameliorate any inflammatory and/or autoimmune diseases attributable to IL-18 signaling. The possible existence of additional affinity-converting subunits for IL-18 can be explored, as well.

20

### SUMMARY OF THE INVENTION

The present invention provides receptor polypeptides designated herein as IL-18 receptor complexes. More particularly, the present invention provides multimeric receptor polypeptides that include an AcPL polypeptide, or fragments thereof, and an IL-1Rrp1 polypeptide, or fragments thereof. The AcPL polypeptide may be covalently  
25 linked or noncovalently to the IL-1Rrp1 polypeptide by any suitable means. Such means include *via* a cross-linking reagent, a polypeptide linker, and associations such as *via* disulfide bonds or by use of leucine zippers. In one embodiment of the invention, the receptor is a fusion protein produced by recombinant DNA technology. This multimeric  
30 receptor of the present invention binds IL-18 with an affinity greater than that of IL-1Rrp1 alone. Disorders mediated by IL-18 may be treated by administering a therapeutically effective amount of this inventive receptor to a patient afflicted with such a disorder.

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### DETAILED DESCRIPTION OF THE INVENTION

The present invention is based upon the discovery that the coexpression of AcPL and IL-1Rrp1 results in a dramatic enhancement of NF $\kappa$ B activity in cells stimulated with IL-18. Because IL-1Rrp1 alone binds IL-18 only weakly and AcPL does not bind IL-18, the enhancement of NF $\kappa$ B activity by coexpressed AcPL and IL-1Rrp1 indicates that  
40 these polypeptides are subunits of an IL-18 receptor complex. In accordance with the present invention novel polypeptides, designated IL-18 receptor complexes, are provided.

5 Advantageously, such dimeric IL-18 receptor complexes comprising IL-1Rrp1 and AcPL, or fragments thereof, are useful for inhibiting IL-18 activity, including the proinflammatory effects of IL-18, and can include IL-1Rrp1 and AcPL as proteins coexpressed in the same cell, or as IL-1Rrp1 linked to an AcPL as receptor subunits. Preferably the subunits are linked via covalent linkages. The subunits may be covalently  
10 linked by any suitable means, such as *via* a cross-linking reagent or a polypeptide linker.

In one embodiment of the present invention, the receptor is a fusion protein produced by recombinant DNA technology. Such fusion proteins can be prepared by transfecting cells with DNA encoding IL-1Rrp1:Fc fusion protein and DNA encoding AcPL:Fc fusion protein and coexpressing the dimers in the same cells.

15 Alternatively, AcPL/IL-1Rrp1 dimers can be prepared by fusing one of the receptor subunits to the constant region of an immunoglobulin heavy chain and fusing the other receptor subunit to the constant region of an immunoglobulin light chain. For example, an AcPL protein can be fused to the CH<sub>1</sub>-hinge-CH<sub>2</sub>-CH<sub>3</sub> region of human IgG1 and an IL-1Rrp1 protein can be fused to the C kappa region of the Ig kappa light chain, or  
20 vice versa. Cells transfected with DNA encoding the immunoglobulin light chain fusion protein and the immunoglobulin heavy chain fusion protein express heavy chain/light chain heterodimers containing the AcPL fusion protein and the IL-1Rrp1 fusion protein. Via disulfide linkages between the heavy chains, the heterodimers further combine to provide multimers, largely tetramers. Advantageously, in the event homodimers of two  
25 heavy or two light chain fusions are expressed, such homodimers can be separated easily from the heterodimers.

In addition to IL-18 receptor protein complexes, the present invention includes isolated DNA encoding heteromer polypeptides, expression vectors containing DNA encoding the heteromer polypeptides, and host cells transformed with such expression  
30 vectors. Methods for production of recombinant IL-18 receptor, including soluble forms of the protein, are also disclosed. Antibodies immunoreactive with the novel polypeptide are provided herein as well.

In one embodiment of the present invention, the polypeptide subunits of the heteromer IL-18 receptors include at least one AcPL subunit as described in SEQ ID  
35 NO:2 or SEQ ID NO: 6, and at least one IL-1Rrp1 subunit as described in SEQ ID NO:4 or SEQ ID NO:8. DNA encoding these polypeptide subunits are presented in SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:3 and SEQ ID NO:7, respectively. The AcPL subunit protein encoded by SEQ ID NO:1 includes an extracellular domain of 356 amino acids (residues 1-356 from N- to C-terminus of SEQ ID NO:2) that includes a signal peptide of  
40 14 amino acids (residues 1-14 of SEQ ID NO:2); a transmembrane region of 25 amino acids (residues 357-381) and a cytoplasmic domain of 218 amino acids (residues 382-

5 599). The AcPL subunit protein encoded by SEQ ID NO:5 includes an extracellular domain of 356 amino acids (residues 1-356 of SEQ ID NO:6) that includes a signal peptide of 14 amino acids (residues 1-14 of SEQ ID NO:6); a transmembrane region of 24 amino acids (residues 357-380) and a cytoplasmic domain of amino acid residues 381-614. The IL-1Rrp1 subunit protein encoded by SEQ ID NO:3 includes an extracellular  
10 domain of 329 amino acids (residues 1-329 of SEQ ID NO:4) that includes a signal peptide of 19 amino acids (residues 1-19 of SEQ ID NO:4); a transmembrane region of 21 amino acids (residues 330 to 350 of SEQ ID NO:4); and, a cytoplasmic domain from amino acid residues 351 to 541. The IL-1Rrp1 subunit protein encoded by SEQ ID NO:7 includes an extracellular domain of 322 amino acids (residues 1-322 of SEQ ID NO:8)  
15 that includes a signal peptide of 18 amino acids (residues 1-18 of SEQ ID NO:8); a transmembrane region of 25 amino acids (residues 323 to 347 of SEQ ID NO:8); and, a cytoplasmic domain from amino acid residues 348 to 537. Additionally, IL-1Rrp1 is described in U.S. Patent No. 5,776,731 and AcPL is described in copending applications S/N 60/078,835 and S/N 60/072,301, incorporated herein by reference.

20 Preferably the polypeptide subunits of the dimeric IL-18 receptors are soluble fragments of IL-1Rrp1 and AcPL polypeptides which together form heteromer complexes having the desired activity. Such polypeptides include those lacking all or part of the transmembrane region and the cytoplasmic domain of the protein. Thus, for example, a heteromer receptor complex of the present invention can include at least one subunit that  
25 is the extracellular domain of SEQ ID NO:2 or SEQ ID NO:6 and at least one subunit that is the extracellular domain of SEQ ID NO:4 or SEQ ID NO:8. These soluble extracellular domains of AcPL and IL-1Rrp1 can include or exclude their signal peptide. Thus, in another embodiment, a heteromeric IL-18 receptor includes amino acid residues 1-356 or residues 15-356 of SEQ ID NO:2 or SEQ ID NO:6, and amino acid residues 1-329 or residues 20-329 of SEQ ID NO:4, or amino acid residues 1-325 or residues 19-322  
30 of SEQ ID NO:8. The desirability of including the signal sequence depends on such factors as the position of the AcPL or IL-1Rrp1 in the fusion protein and the intended host cells when the receptor is to be produced *via* recombinant DNA technology. In preferred embodiments, a DNA construct encoding one of the soluble AcPL or soluble  
35 IL-1Rrp1 fragments is fused to a DNA construct encoding the constant region of an immunoglobulin heavy chain and a DNA construct encoding the other of the soluble AcPL or soluble IL-1Rrp1 fragment is fused to DNA encoding the constant region of an immunoglobulin light chain.

40 Alternatively, the IL-18 receptor may comprise IL-1Rrp1 or soluble IL-1Rrp1 fragments non-covalently complexed with AcPL or soluble AcPL fragments. Non-covalent bonding of IL-1Rrp1 to AcPL may be achieved by any suitable means that does

5 not interfere with the receptor's ability to bind IL-18. In one approach, a first compound is attached to IL-1Rrp1 and a second compound that will non-covalently bond to the first compound is attached to AcPL. Examples of such compounds are biotin and avidin. The receptor is thus formed through the non-covalent interactions of biotin with avidin. In one embodiment of the invention, IL-1Rrp1 and AcPL are recombinant polypeptides,  
10 each purified from recombinant cells and then non-covalently bonded together to form the receptor. A host cell may be transformed with two different expression vectors such that both IL-1Rrp1 and AcPL are produced by the recombinant host cell. IL-1Rrp1 and AcPL produced by such transformed host cells may associate to form a complex through non-covalent interactions. When such transformed cells express the membrane-bound  
15 forms of the proteins, such cells are useful in various assays, including competition assays.

The binding-assay described in example 1 compares the binding of IL-18 by supernatant from cells transfected with IL-1Rrp1 alone, AcPL alone and a combination of IL-1Rrp1 and AcPL. Supernatants from cells coexpressing IL-1Rrp1 and AcPL exhibited  
20 high levels of IL-18 binding; supernatants from cells expressing IL-1Rrp1 alone exhibited low levels of IL-18 binding; and, supernatant from cells transfected with AcPL alone do not bind IL-18. The NF $\kappa$ B induction assay described in example 2 demonstrates that cells transfected with IL-1Rrp1 alone and cells transfected with AcPL alone are not responsive to IL-18 stimulation. However, cells co-transfected with both IL-1Rrp1 and  
25 AcPL and stimulated with IL-18 greatly enhanced NF $\kappa$ B induction..

As used herein, the terms IL-1Rrp1 and AcPL include variants and truncated forms of the native proteins that possess the desired biological activity. Variants produced by adding, substituting, or deleting amino acid(s) in the native sequence are discussed in more detail below.

30 As described above, soluble IL-1Rrp1 and soluble AcPL polypeptides are preferred for certain applications. "Soluble IL-1Rrp1" as used in the context of the present invention refers to polypeptides that are substantially similar in amino acid sequence to all or part of the extracellular region of a native IL-1Rrp1 polypeptide and that, due to the lack of a transmembrane region that would cause retention of the  
35 polypeptide on a cell membrane, are secreted upon expression. Suitable soluble IL-1Rrp1 polypeptides retain the desired biological activity. Soluble IL-1Rrp1 may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble IL-1Rrp1 protein is capable of being secreted.

Likewise, the term "soluble AcPL" as used herein refers to proteins that are  
40 substantially similar in amino acid sequence to all or part of the extracellular region of a native AcPL polypeptide and are secreted upon expression but retain the desired

- 5 biological activity. Soluble AcPL may include part of the transmembrane region, cytoplasmic domain, or other sequences, as long as the polypeptide is secreted.

In one embodiment, soluble IL-1Rrp1 and AcPL polypeptides include the entire extracellular domain. To effect secretion, the soluble polypeptides comprise the native signal peptide or a heterologous signal peptide. Thus, examples of soluble IL-1Rrp1  
10 polypeptides comprise amino acids 1-329 of SEQ ID NO:4 (human IL-1Rrp1) and amino acids 1-322 of SEQ ID NO:8 (murine IL-1Rrp1). Examples of soluble AcPL polypeptides comprise amino acids 1-356 of SEQ ID NO:2 (human AcPL) and amino acids 1-356 of SEQ ID NO:6 (murine AcPL).

A soluble fusion protein comprising the extracellular domain of IL-1Rrp1 of SEQ  
15 ID NO:4 fused to an antibody Fc region polypeptide and the extracellular domain of AcPL fused to an Fc region polypeptide, is described in example 1.

Soluble AcPL and soluble IL-1Rrp1 may be identified (and distinguished from their non-soluble membrane-bound counterparts) by separating intact cells which express the desired protein from the culture medium, e.g., by centrifugation, and assaying the  
20 medium (supernatant) for the presence of the desired protein. The culture medium may be assayed using procedures which are similar or identical to those described in the examples below. The presence of AcPL or IL-1Rrp1 in the medium indicates that the protein was secreted from the cells and thus is a soluble form of the desired protein. Soluble AcPL and soluble IL-1Rrp1 may be naturally-occurring forms of these proteins.  
25 Alternatively, soluble fragments of AcPL and IL-1Rrp1 proteins may be produced by recombinant DNA technology or otherwise isolated, as described below.

The use of soluble forms of IL-1Rrp1 and AcPL is advantageous for certain applications. Purification of the proteins from recombinant host cells is facilitated, since the soluble proteins are secreted from the cells. Further, a receptor of the present  
30 invention comprising soluble IL-1Rrp1 and AcPL proteins is generally more suitable for intravenous administration.

With respect to the foregoing discussion of signal peptides and the various domains of the IL-1Rrp1 and AcPL proteins, the skilled artisan will recognize that the above-described boundaries of such regions of the proteins are approximate. For  
35 example, although computer programs that predict the site of cleavage of a signal peptide are available, cleavage can occur at sites other than those predicted. Further, it is recognized that a protein preparation can comprise a mixture of protein molecules having different N-terminal amino acids, due to cleavage of the signal peptide at more than one site. Thus, soluble IL-1Rrp1 and AcPL polypeptides comprising the extracellular domain  
40 include those having a C-terminal amino acid that may vary from that identified above as the C-terminus of the extracellular domain. Further, post-translational processing that



5 can vary according to the particular expression system employed may yield proteins having differing N-termini. Such variants that retain the desired biological activities are encompassed by the terms "IL-1Rrp1 polypeptides" and "AcPL polypeptides" as used herein.

10 Truncated IL-1Rrp1 and AcPL, including soluble polypeptides, may be prepared by any of a number of conventional techniques. In the case of recombinant proteins, a DNA fragment encoding a desired fragment may be subcloned into an expression vector. Alternatively, a desired DNA sequence may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels.

15 Linkers containing restriction endonuclease cleavage site(s) may be employed to insert the desired DNA fragment into an expression vector, or the fragment may be digested at cleavage sites naturally present therein. Oligonucleotides that reconstruct the N- or C-terminus of a DNA fragment to a desired point may be synthesized. The oligonucleotide may contain a restriction endonuclease cleavage site upstream of the desired coding

20 sequence and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The well known polymerase chain reaction procedure also may be employed to isolate a DNA sequence encoding a desired protein fragment. Oligonucleotide primers comprising the desired termini of the fragment are employed in such a polymerase chain

25 reaction. Any suitable PCR procedure may be employed. One such procedure is described in Saiki et al., *Science* 239:487 (1988). Another is described in *Recombinant DNA Methodology*, Wu et al., eds., Academic Press Inc., San Diego (1989), pp. 189-196. In general, PCR reactions involve combining the 5' and 3' oligonucleotide primers with template DNA (in this case, IL-1Rrp1 or AcPL DNA) and each of the four

30 deoxynucleoside triphosphates, in a suitable buffered solution. The solution is heated, (e.g, from 95°C to 100°C) to denature the double-stranded DNA template and is then cooled before addition of a DNA polymerase enzyme. Multiple cycles of the reactions are carried out in order to amplify the desired DNA fragment.

The AcPL polypeptide is attached to the IL-1Rrp1 polypeptide through a covalent

35 or non-covalent linkage. Covalent attachment is preferred for certain applications, e.g. *in vivo* use, in view of the enhanced stability generally conferred by covalent, as opposed to non-covalent, bonds. In constructing the receptor of the present invention, covalent linkage may be accomplished *via* cross-linking reagents, peptide linkers, or any other suitable technique.

40 Numerous reagents useful for cross-linking one protein molecule to another are known. Heterobifunctional and homobifunctional linkers are available for this purpose

5 from Pierce Chemical Company, Rockford, Illinois, for example. Such linkers contain two functional groups (e.g., esters and/or maleimides) that will react with certain functional groups on amino acid side chains, thus linking one polypeptide to another.

One type of peptide linker that may be employed in the present invention separates AcPL and the IL-1Rrp1 domains by a distance sufficient to ensure that each  
10 domain properly folds into the secondary and tertiary structures necessary for the desired biological activity. The linker also should allow the extracellular domains of AcPL and IL-1Rrp1 to assume the proper spatial orientation to form the binding site for IL-18.

Suitable peptide linkers are known in the art, and may be employed according to conventional techniques. Among the suitable peptide linkers are those described in U.S.  
15 Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A peptide linker may be attached to by any of the conventional procedures used to attach one polypeptide to another. The cross-linking reagents available from Pierce Chemical Company as described above are among those that may be employed. Amino acids having side chains reactive with such reagents may be included in the peptide linker, e.g.,  
20 at the termini thereof. Preferably, a fusion protein comprising AcPL joined to IL-1Rrp1 via a peptide linker is prepared by recombinant DNA technology.

In one embodiment of the invention, AcPL and IL-1Rrp1 are linked via polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising heterologous polypeptides fused to various portions of antibody-derived polypeptides  
25 (including the Fc domain) has been described, e.g., by Ashkenazi et al. (*PNAS USA* 88:10535, 1991) and Byrn et al. (*Nature* 344:677, 1990). As one example, a polypeptide derived from the Fc region of an antibody may be attached to the C-terminus of IL-1Rrp1. A separate Fc polypeptide is attached to the C-terminus of AcPL. Disulfide bonds form between the two Fc polypeptides (e.g., in the so-called hinge region, where  
30 interchain disulfide bonds are normally present in antibody molecules), producing a heterodimer comprising the AcPL/Fc fusion protein linked to the IL-1Rrp1/Fc fusion protein. Advantageously, host cells are co-transfected with two different expression vectors, one encoding soluble IL-1Rrp1/Fc and the other encoding soluble AcPL/Fc. The heterodimer is believed to form intracellularly or during secretion.

35 The term "Fc polypeptide" as used herein includes native and mutein forms, as well as truncated Fc polypeptides containing the hinge region that promotes dimerization. cDNA encoding a single chain polypeptide derived from the Fc region of a human IgG1 antibody can be cloned into the pBluescript SK<sup>®</sup> cloning vector (Stratagene Cloning Systems, LaJolla, CA) to produce a recombinant vector designated hIgG1Fc. A unique  
40 BglII site is positioned near the 5' end of the inserted Fc encoding sequence. An SpeI site is immediately downstream of the stop codon. The Fc polypeptide encoded by the cDNA

5 extends from the N-terminal hinge region to the native C-terminus, i.e., is an essentially full-length antibody Fc region. One suitable mutein of this Fc polypeptide is described in U.S. patent application serial no. 08/097,827, hereby incorporated by reference. The mutein exhibits reduced affinity for Fc receptors.

10 Homodimers comprising two IL-1Rrp1/Fc polypeptides or two AcPL/Fc polypeptides linked via disulfide bonds are also produced by certain of the transfected host cells disclosed herein. The homodimers may be separated from each other and from the heterodimer by virtue of differences in size (e.g., by gel electrophoresis). The heterodimer also may be purified by sequential immunoaffinity chromatography (described below).

15 IL-18 receptor complexes of the present invention include fusion proteins of the constant region of an antibody light chain (or fragment thereof) and the constant region of an antibody heavy chain (or a fragment thereof). The constant region of the heavy chain can include all four of its constant region domains or portion of the domains, including the CH<sub>1</sub> which associates with the light chain, the H hinge region, and the CH<sub>2</sub> and CH<sub>3</sub> domains which are responsible for the dimerization of the heavy chain molecules. Within  
20 the scope of the foregoing fusion proteins are tetramers that are formed by two dimers which link heavy chain/light chain dimers via disulfide linkages between their respective heavy chain regions.

With respect to immunoglobulin light chain polypeptides, polypeptides of the κ family and the λ family are suitable in the practice of this invention. Thus, any type of  
25 immunoglobulin dimer s or tetramer including IgM, IgD, IgG, IgA and IgE can be the basis of the heteromer molecules of the present invention.

In accordance with the present invention, functional heteromeric polypeptides can be prepared by the association between multiple heavy and multiple light chain molecules  
30 which normally associate with one another. For example, the constant region of human IgG1 will associate with the constant region of human light chain κ (designated Cκ). The amino acid sequence of hIgG1 constant region has been reported (Ellison, JW, Berson, BJ and Hood, LE 1982) The nucleotide sequence of a human immunoglobulin C gamma 1 gene is reported. (Nuc. Acids Res. 10: 4071 and Walls, MA, Hsiao, KC and  
35 Harris, LJ 1993). Vectors for the expression of PCR-amplified immunoglobulin variable domains with human constant regions are disclosed. (Nuc. Acids Res. 21:2921) The sequence of human light chain ck has also been reported (Shuford, W, Raff, HV, Finley, JW, Esselstyn, J and Harris, LJ. 1991) Effect of light chain V-region duplication on IgG oligomerization and in vivo efficacy. Science 252:724 and Steinberger, P, Kraft, D and  
40 Valenta, R (1996). Construction of a combinatorial IgE library from an allergic patient.

- 5 Isolation and characterization of human IgE Fabs with specificity for the major timothy grass pollen allergen, Ph1 p 5. J. Biol. Chem., 271:10972).

IL-18 receptor embodiments that include heavy and light chain antibody regions are fusion proteins represented by the formulae:

$$10 \quad \begin{array}{l} R_1-L_1:R_2-L_2 \text{ or } R_2-L_2:R_1-L_1 \text{ or } R_1-L_2:R_2-L_1 \text{ or } R_2-L_1:R_1-L_2 \\ R_1-L_1:R_2-L_2/R_2-L_2:R_1-L_1 \text{ or } R_1-L_2:R_2-L_1/R_2-L_1:R_1-L_2 \end{array}$$

- in which  $L_1$  is an immunoglobulin heavy chain fragment, the N terminus of which extends at least through the  $C_{H1}$  region;  $L_2$  is an immunoglobulin light chain fragment;  $R_1$  is AcPL or an AcPL fragment;  $R_2$  is IL-1Rrp1 or an IL-1Rrp1 fragment; : designates linkages between a heavy chain and light chain antibody region, and / designates linkages between a heavy chain and a heavy chain antibody region. In the case of a dimer, the resulting fusion polypeptide includes two receptor subunits joined by a heavy chain/light chain. In the case of the tetramer, the fusion protein includes four receptor subunits and resembles an antibody in structure, displaying the IL-18 binding site bivalently.

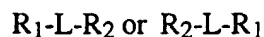
- To obtain the foregoing fusion polypeptides, cDNA encoding an antibody heavy chain polypeptide derived from human IgG1 antibody ( $CH_1-H-CH_2-CH_3$ ) can be cloned into the pDC409 expression vector to produce a recombinant vector designated hIgG1. A unique BglII site is positioned near the 5' end of the inserted heavy chain encoding sequence. A NotI site is immediately downstream of the stop codon. The heavy chain polypeptide, encoded by the cDNA extends from the N-terminus of the  $CH_1$  region to the native C-terminus. To obtain an antibody light chain cDNA encoding a single chain polypeptide derived from the human kappa chain constant regions can be cloned in the pDC409 expression vector to produce a recombinant vector designated hIgk. This sequence is flanked at the 5' end by a unique BglII site and at the 3' end by a unique NotI site. Embodiments of the present invention that incorporate such antibody polypeptides include a first fusion polypeptide comprising AcPL (or a fragment thereof) upstream of the constant region of an antibody light chain (or a fragment thereof) and a second fusion polypeptide comprising IL-1Rrp1 upstream of the constant region of an antibody heavy chain (or a heavy chain fragment), the N-terminus of which extends at least through the  $CH_1$  region. Disulfide bond(s) form between the AcPL light chain fusion polypeptide and the IL-1Rrp1-heavy chain fusion polypeptide, thus producing a receptor of the present invention. As a further alternative, an IL-1Rrp1-antibody light chain fusion polypeptide is prepared and combined with (disulfide bonded to) a fusion polypeptide comprising AcPL-antibody heavy chain fusion polypeptide. When two of the foregoing disulfide bonded molecules are combined, additional disulfide bonds form between the two

5 antibody regions. The resulting receptor of the present invention comprising four fusion polypeptides resembles an antibody in structure and displays the IL-18 binding site bivalently.

The AcPL and IL-1Rrp1 polypeptides may be separately purified from cellular sources, and then linked together. Alternatively, the receptor of the present invention  
10 may be produced using recombinant DNA technology. The AcPL and IL-1Rrp1 polypeptides may be produced separately and purified from transformed host cells for subsequent covalent linkage. In one embodiment of the present invention, a host cell is transformed/transfected with foreign DNA that encodes AcPL and IL-1Rrp1 as separate polypeptides. The two polypeptides may be encoded by the same expression vector with  
15 start and stop codons for each of the two genes, or the recombinant cells may be co-transfected with two separate expression vectors. In another embodiment, the receptor is produced as a fusion protein in recombinant cells.

In one embodiment of the present invention, the receptor protein is a recombinant fusion protein of the formula:

20



wherein  $R_1$  represents AcPL or an AcPL fragment;  $R_2$  represents IL-1Rrp1 or an IL-1Rrp1 fragment; and L represents a peptide linker.

25 The fusion proteins of the present invention include constructs in which the C-terminal portion of AcPL is fused to the linker which is fused to the N-terminal portion of IL-1Rrp1, and also constructs in which the C-terminal portion of IL-1Rrp1 is fused to the linker which is fused to the N-terminal portion of AcPL. AcPL is covalently linked to IL-1Rrp1 in such a manner as to produce a single protein which retains the desired biological  
30 activities of AcPL and IL-1Rrp1. The components of the fusion protein are listed in their order of occurrence (i.e., the N-terminal polypeptide is listed first, followed by the linker and then the C-terminal polypeptide).

A DNA sequence encoding a fusion protein is constructed using recombinant DNA techniques to insert separate DNA fragments encoding AcPL and IL-1Rrp1 into an  
35 appropriate expression vector. The 3' end of a DNA fragment encoding AcPL is ligated (via the linker) to the 5' end of the DNA fragment encoding IL-1Rrp1 with the reading frames of the sequences in phase to permit translation of the mRNA into a single biologically active fusion protein. Alternatively, the 3' end of a DNA fragment encoding IL-1Rrp1 may be ligated (via the linker) to the 5' end of the DNA fragment encoding  
40 AcPL, with the reading frames of the sequences in phase to permit translation of the mRNA into a single biologically active fusion protein. A DNA sequence encoding an N-

5 terminal signal sequence may be retained on the DNA sequence encoding the N-terminal polypeptide, while stop codons, which would prevent read-through to the second (C-terminal) DNA sequence, are eliminated. Conversely, a stop codon required to end translation is retained on the second DNA sequence. DNA encoding a signal sequence is preferably removed from the DNA sequence encoding the C-terminal polypeptide.

10 A DNA sequence encoding a desired polypeptide linker may be inserted between, and in the same reading frame as, the DNA sequences encoding AcPL and IL-1Rrp1 using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker and containing appropriate restriction endonuclease cleavage sites may be ligated between the sequences encoding AcPL and IL-1Rrp1.

15 Alternatively, a chemically synthesized DNA sequence may contain a sequence complementary to the 3' terminus (without the stop codon) of either AcPL and IL-1Rrp1, followed by a linker-encoding sequence which is followed by a sequence complementary to the 5' terminus of the other of AcPL and IL-1Rrp1. Oligonucleotide directed mutagenesis is then employed to insert the linker-encoding sequence into a vector  
20 containing a direct fusion of AcPL and IL-1Rrp1.

The present invention provides isolated DNA sequences encoding the above-described fusion proteins comprising AcPL, IL-1Rrp1, and a peptide linker. DNA encoding AcPL polypeptides disclosed herein is also provided, as is DNA encoding AcPL polypeptides fused to immunoglobulin-derived polypeptides. AcPL-encoding  
25 DNA encompassed by the present invention includes, for example, cDNA, chemically synthesized DNA, DNA isolated by PCR, genomic DNA, and combinations thereof.

Also provided herein are recombinant expression vectors containing the isolated DNA sequences. "Expression vector" refers to a replicable DNA construct used to express DNA which encodes the desired protein and which includes a transcriptional unit  
30 comprising an assembly of (1) genetic element(s) having a regulatory role in gene expression, for example, promoters, operators, or enhancers, operatively linked to (2) a DNA sequence encoding a desired protein which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. The choice of promoter and other regulatory elements generally varies  
35 according to the intended host cell.

In the expression vectors, regulatory elements controlling transcription or translation are generally derived from mammalian, microbial, viral or insect genes. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. Vectors  
40 derived from retroviruses also may be employed.

5 DNA regions are operably linked when they are functionally related to each other. For example, DNA encoding a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if the polypeptide is expressed as a precursor that is secreted through the host cell membrane; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; and a ribosome binding site is operably linked  
10 to a coding sequence if it is positioned so as to permit translation. Generally, "operably linked" means contiguous and, in the case of secretory leaders, contiguous and in reading frame.

Transformed host cells are cells which have been transformed or transfected with foreign DNA using recombinant DNA techniques. In the context of the present  
15 invention, the foreign DNA includes a sequence encoding the inventive proteins. Host cells may be transformed for purposes of cloning or amplifying the foreign DNA, or may be transformed with an expression vector for production of the protein. Suitable host cells include prokaryotes, yeast or higher eukaryotic cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are  
20 described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic  
25 resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Examples of suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

30 Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322  
35 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and this provides simple means for identifying transformed cells.

40 Promoters commonly used in recombinant microbial expression vectors include the  $\beta$ -lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature*

5 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), the tryptophan (*trp*) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 36,776) and *tac* promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage  $\lambda$  P<sub>L</sub> promoter and cI857ts thermoinducible repressor. Plasmid vectors available from the  
10 American Type Culture Collection which incorporate derivatives of the  $\lambda$  P<sub>L</sub> promoter include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

The recombinant receptor protein may also be expressed in yeast hosts, preferably from *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera such as *Pichia*  
15 or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2 $\mu$ m yeast plasmid or an autonomously replicating sequence (ARS), a promoter, DNA encoding the receptor fusion protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable markers permitting  
20 transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and the *S. cerevisiae* *trp1* gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for  
25 detecting transformation by growth in the absence of tryptophan.

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate  
30 dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for  
35 selection and replication in *E. coli* (Amp<sup>r</sup> gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and  $\alpha$ -factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al., (*Nature* 300:724, 1982). The yeast  $\alpha$ -factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the  
40 structural gene to be expressed. See, e.g., Kurjan et al., *Cell* 30:922, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to



5 contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

Suitable yeast transformation protocols are known to those of skill in the art. An exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, (1978), selecting for Trp<sup>+</sup> transformants in a selective medium consisting of 0.67% yeast  
10 nitrogen base, 0.5% casamino acids, 2% glucose, 10 µg/ml adenine and 20 µg/ml uracil.

Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 µg/ml adenine and 80 µg/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast  
15 supernatants are harvested by filtration and held at 4°C prior to further purification.

Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Examples of suitable mammalian host cell lines include L cells, C127, 3T3, Chinese  
20 hamster ovary (CHO), HeLa, and BHK cell lines. Additional suitable mammalian host cells include CV-1 cells (ATCC CCL70) and COS-7 cells (ATCC CRL 1651; described by Gluzman, *Cell* 23:175, 1981), both derived from monkey kidney. Another monkey kidney cell line, CV-1/EBNA (ATCC CRL 10478), was derived by transfection of the CV-1 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) and  
25 with a vector containing CMV regulatory sequences (McMahan et al., *EMBO J.* 10:2821, 1991). The EBNA-1 gene allows for episomal replication of expression vectors, such as HAV-EO or pDC406, that contain the EBV origin of replication.

Mammalian expression vectors may comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be  
30 expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a poly-adenylation site, splice donor and acceptor sites, and transcriptional termination sequences. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and  
35 enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained  
40 easily from the virus as a fragment which also contains the SV40 viral origin or replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may

5 also be used, provided the approximately 250 bp sequence extending from the Hind III site toward the *BglII* site located in the viral origin of replication is included.

Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983). One useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be  
10 constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). Vectors derived from retroviruses also may be employed.

When secretion of the AcPL and/or IL-1Rrp1 protein from the host cell is desired, the expression vector may comprise DNA encoding a signal or leader peptide. In place of the native signal sequence, a heterologous signal sequence may be added, such as the  
15 signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman et al., *Nature* 312:768 (1984); the interleukin-4 signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

20 The present invention provides a process for preparing the recombinant proteins of the present invention, comprising culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes said protein under conditions that promote expression. The desired protein is then purified from culture media or cell extracts. The desired protein may be AcPL, IL-1Rrp1 or the heterodimeric receptor, for  
25 example. Cell-free translation systems could also be employed to produce the desired protein using RNA derived from the novel DNA of the present invention.

As one example, supernatants from expression systems that secrete recombinant protein into the culture medium can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration  
30 unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise IL-18. An IL-18 affinity matrix may be prepared by coupling recombinant human IL-18 to cyanogen bromide-activated Sepharose (Pharmacia) or Hydrazide Affigel (Biorad), according to manufacturer's recommendations. Sequential immunopurification using antibodies bound  
35 to a suitable support is preferred. Proteins binding to an antibody specific for AcPL are recovered and contacted with antibody specific for IL-1Rrp1 on an insoluble support. Proteins immunoreactive with both antibodies may thus be identified and isolated.

Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be  
40 acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation

5     exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. One or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a fusion protein.

10     Some or all of the foregoing purification steps, in various combinations, can be employed to provide an essentially homogeneous recombinant protein. Recombinant cell culture enables the production of the fusion protein free of those contaminating proteins which may be normally associated with IL-1Rrp1 or AcPL as they are found in nature in their respective species of origin, e.g., on the surface of certain cell types.

15     The foregoing purification procedures are among those that may be employed to purify non-recombinant receptors of the present invention as well. When linking procedures that may produce homodimers (IL-1Rrp1-linker-IL-1Rrp1 and AcPL-linker-AcPL) are employed, purification procedures that separate the heterodimer from such homodimers are employed. An example of such a procedure is sequential  
20     immunopurification as discussed above. In one embodiment, AcPL (recombinant or non-recombinant) is purified such that no bands corresponding to other (contaminating) proteins are detectable by SDS-PAGE.

25     Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant fusion proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

30     Fermentation of yeast which express fusion proteins as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984), involving two sequential, reversed-phase HPLC steps for purification of a recombinant protein on a preparative HPLC column.

35     The DNA or amino acid sequences of IL-1Rrp1 or AcPL may vary from those presented in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7. Due to the known degeneracy of the genetic code, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. In addition, DNA sequences capable of hybridizing to the native DNA sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID  
40     DNO:5 or SEQ ID NO:7 under moderately stringent or highly stringent conditions, and which encode a biologically active IL-1Rrp1 or AcPL polypeptide, are also considered to

5 be IL-1Rrp1-encoding or AcPL-encoding DNA sequences, in the context of the present invention. Such hybridizing sequences include but are not limited to variant sequences such as those described below, and DNA derived from other mammalian species.

Moderately stringent conditions include conditions described in, for example, Sambrook et al, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Vol. 1, pp 1.101-  
10 104, Cold Spring Harbor Laboratory Press, 1989. Conditions of moderate stringency, as defined by Sambrook et al., include use of a prewashing solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of about 55°C, 5 X SSC, overnight. Highly stringent conditions include higher temperatures of hybridization and washing. The skilled artisan will recognize that the temperature and wash solution salt  
15 concentration may be adjusted as necessary according to factors such as the length of the probe., wherein said conditions include hybridization at 68°C followed by washing in 0.1X SSC/0.1% SDS at 63-68°C. In another embodiment, the present invention provides a heterodimeric receptor comprising AcPL and IL-1Rrp1, wherein the AcPL and the IL-1Rrp1 are encoded by DNA that hybridizes to the DNA of SEQ ID NO:1 or SEQ ID  
20 NO:5, or SEQ ID NO:3 or SEQ ID NO:7, respectively, under moderately or highly stringent conditions.

Further, certain mutations in a nucleotide sequence which encodes AcPL or IL-1Rrp1 will not be expressed in the final protein product. For example, nucleotide  
substitutions may be made to enhance expression, primarily to avoid secondary structure  
25 loops in the transcribed mRNA (see EP 75,444A). Other alterations of the nucleotide sequence may be made to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

The amino acid sequence of native IL-1Rrp1 or AcPL may be varied by substituting, deleting, adding, or inserting one or more amino acids to produce a IL-  
30 1Rrp1 or AcPL variant. Variants that possess the desired biological activity of the native IL-1Rrp1 and AcPL proteins may be employed in the receptor of the present invention. Assays by which the biological activity of variant proteins may be analyzed are described in the examples below. Biologically active IL-1Rrp1 polypeptides are capable of binding IL-18. The desired biological activity of the AcPL polypeptides disclosed herein is the  
35 ability to enhance the binding of IL-18 when AcPL is joined to IL-1Rrp1, compared to the level of IL-18 binding to IL-1Rrp1 alone.

Alterations to the native amino acid sequence may be accomplished by any of a number of known techniques. For example, mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction  
40 sites enabling ligation to fragments of the native sequence. Following ligation, the

5 resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the  
10 alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craig (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); U.S. Patent No. 4,518,584, and U.S. Patent No. 4,737,462, which are incorporated by reference herein.

Bioequivalent variants of AcPL and IL-1Rrp1 may be constructed by, for  
15 example, making various substitutions of amino acid residues or deleting terminal or internal amino acids not needed for biological activity. In one embodiment of the invention, the variant amino acid sequence is at least 80% identical, preferably at least 90% identical, to the native sequence. Percent similarity may be determined, for example, by comparing sequence information using the GAP computer program, version  
20 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443, 1970), as revised by Smith and Waterman (*Adv. Appl. Math.* 2:482, 1981). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in  
25 the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp.  
30 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those having physiochemical characteristics resembling those of the residue to be replaced. Examples of conservative substitutions include substitution  
35 of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Cysteine residues can be deleted or replaced with other amino acids to prevent  
40 formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. Hydrophilic amino acids may be substituted for hydrophobic amino acids in the

- 5 transmembrane region and/or intracellular domain of IL-1Rrp1 and AcPL to enhance water solubility of the proteins.

Adjacent dibasic amino acid residues may be modified to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein.

10 KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. These amino acid pairs, which constitute KEX2 proteases processing sites, are found at residues 98-99, 323-333, 333-334, 472-473 and 475-476 of the AcPL protein of SEQ ID NO:2. These KEX2 sites are found at positions 113-114,

15 314-315, 364-365, 437-438, and 465-466 of the IL-1Rrp1 protein of SEQ ID NO:4. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

The present invention also includes proteins with or without associated native-pattern glycosylation. Expression of DNAs encoding the fusion proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast

20 expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A<sub>1</sub>-Z, where A<sub>1</sub> is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate.

The AcPL amino acid sequence in SEQ ID NO:2 contains 4 such N-glycosylation sites, all found in the extracellular domain, at amino acids 21-23, 119-121, 152-254 and 345-347. The extracellular domain of IL-1Rrp1 comprises N-glycosylation sites at positions 91-93, 102-104, 150-153, 168-170, 197-199, 203-205, 236-238, and 297-299 of SEQ ID NO:4. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A<sub>1</sub> and Z, or

30 an amino acid other than Asn between Asn and A<sub>1</sub>. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 276,846.

Variants of the receptor proteins of the present invention also include various structural forms of the primary protein which retain biological activity. Due to the

40 presence of ionizable amino and carboxyl groups, for example, a receptor protein may be

5 in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure also may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives are prepared by linking  
10 particular functional groups to amino acid side chains or at the N- or C- termini. Other derivatives of the receptor protein within the scope of this invention include covalent or aggregative conjugates of the receptor protein with other proteins or polypeptides, such as by synthesis in recombinant culture as N- or C- terminal fusions. For example, the conjugated polypeptide may be a signal (or leader) polypeptide sequence at the N-  
15 terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast  $\alpha$ -factor leader).

Peptides may be fused to the desired protein (e.g., *via* recombinant DNA techniques) to facilitate purification or identification. Examples include poly-His or the  
20 Flag<sup>®</sup> peptide (Hopp et al., *Bio/Technology* 6:1204, 1988, and U.S. Patent 5,011,912). The Flag<sup>®</sup> peptide is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. Expression systems useful for fusing the Flag<sup>®</sup> octapeptide to the N- or C-terminus of a given protein are available from Eastman Kodak Co., Scientific  
25 Imaging Systems Division, New Haven, CT, as are monoclonal antibodies that bind the octapeptide.

Dimer IL-18 receptor complexes that include naturally occurring variants of IL-1Rrp1 and AcPL are also encompassed by the present invention. Examples of such variants are proteins that result from alternative mRNA splicing events or from  
30 proteolytic cleavage of the AcPL and IL-1Rrp1 protein, wherein the desired biological activity is retained. Alternative splicing of mRNA may yield a truncated but biologically active AcPL and IL-1Rrp1 protein, such as a naturally occurring soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C- termini upon expression in different types of host cells, due to proteolytic removal  
35 of one or more terminal amino acids from the AcPL or IL-1Rrp1 protein (generally from 1-5 terminal amino acids).

The present invention also provides a pharmaceutical composition comprising a receptor protein of the present invention with a physiologically acceptable carrier or diluent. Such carriers and diluents will be nontoxic to recipients at the dosages and  
40 concentrations employed. Such compositions may, for example, comprise the receptor protein in a buffered solution, to which may be added antioxidants such as ascorbic acid,

5 low molecular weight (less than about ten residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. The receptor of the present invention may be administered by any suitable method in a manner appropriate to the indication, such as intravenous injection, local administration, continuous infusion, sustained release  
10 from implants, etc.

Heterodimeric receptors of the present invention are useful as an IL-18 binding reagent. This receptor, which preferably comprises soluble AcPL and soluble IL-1Rrp1, has applications both *in vitro* and *in vivo*. The receptors may be employed in *in vitro* assays, e.g., in studies of the mechanism of transduction of the biological signal that is  
15 initiated by binding of IL-18 to this receptor on a cell. Such receptors also could be used to inhibit a biological activity of IL-18 in various *in vitro* assays or *in vivo* procedures. In one embodiment of the invention, the inventive receptor is administered to bind IL-18, thus inhibiting binding of the IL-18 to endogenous cell surface receptors. Biological activity mediated by such binding of IL-18 to the cells thus is also inhibited.

20 IL-1Rrp1 alone binds IL-18, but with relatively low affinity (Torigoe et al. *J. Biol. Chem.* 272:2573, 1997). Receptors of the present invention, produced by cells co-transfected with AcPL and IL-1Rrp1-encoding DNA, for example, bind IL-18 with high affinity. Such receptors of the present invention may be employed when inhibition of an IL-18-mediated activity is desired. In addition, use of the receptors of the present  
25 invention *in vitro* assays offers the advantage of allowing one to determine that the assay results are attributable to binding of IL-18.

In one embodiment of the invention, a heterodimeric receptor comprising AcPL and IL-1Rrp1 is administered *in vivo* to inhibit a biological activity of IL-18. IL-18 is known to mediate NF $\kappa$ B activity and acts as a stimulator of Th1 cell growth and  
30 differentiation, and is a potent inducer of  $\gamma$ -interferon production from Th1 cells. IL-18 also enhances NK cell killing activity and has been implicated in septic shock, liver destruction, and diabetes. When these or other biological effects of IL-18 are undesirable, a receptor of the present invention may be administered to bind IL-18 and ameliorate the effects of IL-18 activity.

35 The inventive receptor may be administered to a patient in a therapeutically effective amount to treat a disorder mediated by IL-18. A disorder is said to be mediated by IL-18 when IL-18 causes (directly or indirectly) or exacerbates the disorder. Soluble receptor proteins can be used to competitively bind to IL-18, thereby inhibiting binding of IL-18 to endogenous cell surface receptors.

40 Heterodimeric receptors comprising AcPL linked to IL-1Rrp1 also find use in assays for biological activity of IL-18 proteins, which biological activity is measured in



5 terms of binding affinity for the receptor. To illustrate, the receptor may be employed in a binding assay to measure the biological activity of an IL-18 fragment, variant, or mutein. The receptor is useful for determining whether biological activity of IL-18 is retained after modification of an IL-18 protein (e.g., chemical modification, mutation, etc.). The binding affinity of the modified IL-18 protein for the receptor is compared to  
10 that of an unmodified IL-18 protein to detect any adverse impact of the modification on biological activity. Biological activity thus can be assessed before the modified protein is used in a research study or assay, for example.

The heterodimeric receptors also find use as reagents that may be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of IL-  
15 18 proteins under different conditions. The receptors may be used to confirm biological activity (in terms of binding affinity for the receptor) in IL-18 proteins that have been stored at different temperatures, for different periods of time, or which have been produced in different types of recombinant expression systems, for example.

The following examples are provided to illustrate certain embodiments of the  
20 invention, and are not to be construed as limiting the scope of the invention.

## **EXAMPLES**

### **Example 1**

#### **In Vitro Precipitation Experiments**

In order to determine whether AcPL, IL-1Rrp1 or combinations of the two polypeptides bind IL-18, several Fc fusion proteins were prepared and tested as follows. An expression vector encoding a soluble AcPL/Fc fusion protein, which comprised a  
30 truncated extracellular domain of AcPL fused to the N-terminus of an Fc region polypeptide derived from an antibody, was constructed as follows. A recombinant expression vector comprising AcPL DNA in vector pDC304, was PCR amplified utilizing primers containing the desired in-frame restriction sites on the 5' and 3' ends. The resulting fragment, which includes the 5' end of the AcPL DNA, terminating at  
35 nucleotide 1551 of SEQ ID NO:1, with introduced SalI and BglII sites at the 5' and 3' ends, respectively, was isolated, by conventional techniques.

A recombinant vector designated pDC412-hIgG1Fc comprises the Fc polypeptide-encoding cDNA (-H-CH<sub>2</sub>-CH<sub>3</sub> only). Vector pDC412-hIgG1Fc was digested with the restriction enzymes SalI and BglII, which cleave in the polylinker region of the  
40 vector, upstream of the Fc polypeptide-encoding cDNA.

5 The AcPL-encoding DNA fragment isolated above was ligated into a SalI/BglII-digested pDC412-hIgG1Fc such that the Fc polypeptide DNA was fused to the 3' end of the AcPL DNA. The resulting expression vector encoded a fusion protein comprising amino acids 1-356 of the AcPL sequence of SEQ ID NO:2, followed by the H-CH<sub>2</sub>-CH<sub>3</sub> region of hIgG1Fc.

10 An expression vector encoding a soluble human IL-1Rrp1/Fc fusion protein was constructed as follows. A recombinant vector that includes IL-1Rrp1 cDNA was PCR amplified with gene-specific primers containing the desired restriction sites. The resulting fragment, which includes the 5' end of IL-1Rrp1 was isolated by conventional techniques. This IL-1Rrp1 fragment, digested with Asp718 and BglII, was combined  
15 with the hIgG1Fc fragment described above and digested with BglII and NotI. The resulting digest fragments were ligated to pDC304 digested with Asp718 and Not I. The resulting IL-1Rrp1/Fc fusion protein encoded by the recombinant vector comprises (from N- to C-terminus) amino acids 1-329 of SEQ ID NO:4, followed by the H-CH<sub>2</sub>-CH<sub>3</sub> region of hIgG1/Fc.

20 In one sample set, COS-7 cells were transfected with a pDC206 control or pDC206-IL-18 vector. In another sample set of transfected COS-7 cells, the Fc fusion vectors described above were transfected. The total sample set was as follows:

	<u>Sample</u>	<u>Cells transfected with vector(s) encoding:</u>
25	A	empty pDC206 expression vector (control)
	B	pDC206hIL-18
	1	pDC409 (control)
	2	IL-1Rrp1/Fc
	3	AcPL/Fc
30	4	AcPL/Fc and IL-1Rrp1/Fc

Two days post transfection, samples A and B were starved 1 hour in cys/met-free medium, then labeled with [<sup>35</sup>S-cys][<sup>35</sup>S-met]-containing medium for 6 hours. Supernatants were removed, subjected to centrifugation, and adjusted to 0.4M NaCl/1.0% Triton X-100 in the presence of protease inhibitors. The supernatants from cells  
35 transfected with the Fc fusion proteins of Samples 1-4 were removed at 2 days post transfection and centrifuged. Each Fc fusion supernatant was combined with either a) vector-transfected: or, b) IL-18 transfected <sup>35</sup>S-labeled supernatants. Purified IL-1Rrp1/Fc-receptor protein was added to another portion of the radiolabeled supernatant as  
40 a control. Protein G-Sepharose was added to each experimental sample and precipitations were carried out overnight at 4°C. Then the samples were washed

5 extensively in a 0.4M NaCl, 0.05% SDS, 1.0% NP-40 buffer and separated by electrophoresis in a 4-20% Tris-Glycine gel. The gel was fixed, amplified, dried and exposed to film. In order to assess total levels of protein and take into account unlabeled Fc-fusion proteins, a portion of each precipitate was analyzed on a separate 4-20% Tris-Glycine gel and silver stained.

10 Supernatants from cell samples 1-4 did not noticeably precipitate any proteins in the 10-30 Kd range from the control supernatant (Sample A). IL-18 (Sample B) was precipitated by supernatant from cell sample 2 (IL-1Rrp1Fc) but not by supernatant from cell sample 1 (control) or cell sample 3 (AcPL/Fc). Significantly more IL-18 was precipitated by supernatant from all sample 4 that was obtained from the cotransfection of  
15 IL-1Rrp1/Fc and AcPL/Fc.

Thus, IL-1Rrp1 is able to bind IL-18; AcPL is not able to bind IL-18; and, coexpressed IL-1Rrp1 and AcPL are able to bind IL-18 and the coexpressed proteins exhibit higher levels of binding of IL-18 than IL-1Rrp1 alone. The silver stained gel shows that there is no more IL-1Rrp1 in supernatants transfected with IL-1Rrp1 and AcPL  
20 as compared to supernatants transfected with IL-1Rrp1 alone. This rules out the possibility that there is more IL-1Rrp1 expressed in these samples. The results indicate that the IL-18 binding affinity of an IL-1Rrp1/AcPL dimer is greater than the affinity of IL-1Rrp1 alone.

### Example 2

#### 25 Induction of NFκB Activity

In order to determine the roles of IL-1Rrp1 and AcPL in IL-18 signaling, AcPL, IL-1Rrp1, and a combination of IL-1Rrp1 and AcPL were overexpressed in COS cells and S49.1 cells and the effect of IL-18 stimulation on NFκB activation was assessed.

30 COS-7 cells were transfected by the DEAE/Dextran method in a 12-well format. Each well was transfected with a total of 200 ng of the appropriate expression vector(s) and 800ng of a NFκB-Luc reporter plasmid, which contains 3 NFκB sites mediating luciferase expression. Approximately  $10^7$  S49.1 cells were transfected by electroporation in 0.7 mL with 40μg of the NFκB-Luc reporter plasmid, and a total of 20μg of the  
35 appropriate expression vector(s). Electroporations were performed at 960μF and 320V.

The cells were incubated for 2 days, and then stimulated with 40 ng/mL of IL-18 (purchased from PeproTech) for 4 hours. Cells were washed, lysed, and assayed for luciferase activity using Luciferase Assay Reagents (purchased from Promega Corp.) according to the manufacturer's instructions.

40 COS7 or S49.1 cells that were transfected with control vector alone, vector encoding mIL-1Rrp1 alone, or vector encoding mAcPL alone were not responsive to

5 mIL-18 stimulation. Furthermore, S49.1 cells transfected with mAcPL were not responsive to mIL-18 signaling when the transfection was in combination with an expression vector encoding mIL-1R type I or mIL-1RAcP. However, cells cotransfected with mAcPL and mIL-1Rrp1 and stimulated with mIL-18 showed an increase in NFκB DNA binding activity that was 10 fold in COS cells and 300 fold in S49.1 cells. COS7  
10 cells transfected with hIL-1Rrp1 displayed no response to hIL-18 stimulation, while COS7 cells transfected with hAcPL alone and stimulated with hIL-18 showed an 8 fold increase in NFκB activity. This is attributed to the association of hAcPL with monkey IL-1Rrp1 endogenous to COS7 cells. Overexpression of hIL-1Rrp1 with hAcPL did not augment the stimulation of NFκB activity in response to hIL-18 over that seen in cells  
15 overexpressing hAcPL alone. This dramatic enhancement of NFκB activity indicates that AcPL and IL-1Rrp1 are subunits of the IL-18 receptor and cooperate to induce NFκB signaling in response to IL-18 stimulation.

### EXAMPLE 3

#### 20 Preparing AcPL and IL-1Rrp1 Antibody Heavy and Light Chain Fusion Proteins

The following describes preparing fusion proteins that include AcPL and IL-1Rrp1 fused to an antibody heavy chain and antibody light chain polypeptide.

First, an expression vector encoding the entire constant region of human IgG1 with a linker region upstream is constructed. Such an expression vector facilitates the  
25 creation of fusion protein-encoding plasmids. PCR techniques are utilized to amplify the above mentioned IgG1 constant region with primers containing an upstream BglII site and a downstream NotI site. The resulting PCR generated fragment is digested, purified, and ligated to pDC412 which is digested with BglII and NotI. The pDC412-hIgG1 expression vector is then digested with SalI and BglII.

30 Next an expression vector containing the Ig κ constant domain preceded by a linker region and followed by a linker region and poly-His domain is prepared. The poly-His domain advantageously aids in the protein purification process. PCR techniques are utilized to amplify the constant region with primers containing a BglII-NotI fragment. The resulting PCR generated fragment is digested, purified, and ligated to pDC412.  
35 Soluble receptors of interest can be cloned upstream by utilizing the unique SalI and BglII sites.

To prepare an IL-1Rrp1-Cκ expression vector, the extracellular domain of IL-1Rrp1 is PCR amplified using primers containing SalI (5') and BglII (3') restriction sites. This purified and digested PCR product is ligated to SalI/BglII digested the pDC412-Ig κ  
40 expression vector to create an in-frame construct that encodes a fusion protein linking the soluble portion of IL-1Rrp1 to the constant region of Cκ.

- 5 To prepare an IL-1Rrp1-hIgG1 expression vector the SalI/BglII restriction fragment encoding soluble IL-1Rrp1 is removed from IL-1Rrp1-Ck and ligated to pDC412-hIgG1 which has been digested with the same restriction enzymes. Since both vectors contain the BglII site in the same reading frame, this will readily generate a fusion between soluble IL-1Rrp1 and hIgG1.
- 10 To prepare an AcPL-Ck expression vector, the extracellular domain of AcPL is PCR amplified using primers containing SalI (5') and BglII (3') restriction sites. This purified and digested PCR product then is ligated to SalI/BglII digested pDC412-Ck to create an in-frame fusion protein linking the soluble portion of AcPL to the constant region of Ck.
- 15 To prepare an AcPL-hIgG1 expression vector the SalI/BglII restriction fragment encoding soluble AcPL is removed from AcPL-ck and ligated to pDC412-hIgG1 which has been digested with the same restriction enzymes. Since both vectors contain the BglII site in the same reading frame, this will readily generate a fusion between soluble AcPL and hIgG1.
- 20 COS-7 cells are transfected with the above described fusion vectors. The cells are cultured and the fusion proteins are collected as described in Example 1.

#### EXAMPLE 4

##### Inhibition of An IL-18 Induced NFkB Activity

- 25 Cos7 cells were transiently transfected in 12-well plates with 10 ng each of mIL-1Rrp1 and mAcPL expression vectors, and 50 ng of a 3XNFkB-Luciferase reporter plasmid per well. Two days post-transfection, cells were stimulated with 10 ng/ml mIL-18 (purchased from Peprotech) in the presence of increasing amounts of various receptor-Fc fusion proteins. mIL-18 was preincubated with the proteins for 20 min at room
- 30 temperature prior to addition to cells. The amount of Fc protein was titrated from 1 ug/ml to 50 ug/ml. Cells were stimulated 4 hours, then lysed and luciferase activity was assessed using the Promega Luciferase Assay Reagents.

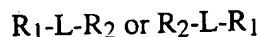
- Preincubation of IL-18 with mIL-1Rrp1-Fc, mIL-1Rrp1-FlagpolyHis, or mAcPL-Fc had no significant effect on induction of NFkB at any of the concentrations tested. In
- 35 contrast, incubation of IL-18 with a heterogeneous mIL-1Rrp1-Fc + mAcPL-Fc protein mixture (consisting of homodimers of mIL-1Rrp1-Fc, homodimers of mAcPL-Fc, and heterodimeric mIL-1Rrp1-Fc/mAcPL-Fc molecules) resulted in a dose-dependent inhibition of NFkB induction. Uninduced cells displayed 3 X 10<sup>3</sup> RLU, and cells stimulated with mIL-18 in the absence of any receptor-Fc protein displayed 25 X 10<sup>3</sup>
- 40 RLU. Maximal inhibition of NFkB induction was observed with 20 ug/ml and 50 ug/ml

- 5 of mIL-1R-rp1-Fc + mAcPL-Fc protein mixture, which resulted in  $6 \times 10^3$  RLU, representing an 87% inhibition of IL-18 activity.

## CLAIMS

What is claimed is:

1. A receptor comprising at least one IL-1Rrp1 polypeptide linked to at least one AcPL polypeptide, wherein said IL-1Rrp1 polypeptide is encoded by a DNA selected from the group consisting of:
  - a) DNA comprising a nucleotide sequence selected from the group consisting of the coding region of the nucleotide sequence presented in SEQ ID NO:3 and the coding region of the nucleotide sequence presented in SEQ ID NO: 7;
  - b) DNA capable of hybridizing under highly stringent conditions to the DNA of (a); and
  - c) DNA selected from the group consisting of: DNA that encodes the amino acid sequence presented in SEQ ID NO:4 and DNA that encodes the amino acid sequence presented in SEQ ID NO:8; andwherein said AcPL polypeptide is a biologically active polypeptide encoded by a DNA selected from the group consisting of:
  - a') DNA comprising a nucleotide sequence selected from the group consisting of the coding region of the nucleotide sequence presented in SEQ ID NO:1 and the coding region of the nucleotide sequence presented in SEQ ID NO: 5;
  - b') DNA capable of hybridizing under highly stringent conditions to the DNA of (a'); and
  - c') DNA selected from the group consisting of: DNA that encodes the amino acid sequence presented in SEQ ID NO:2 and DNA that encodes the amino acid sequence presented in SEQ ID NO:6.
2. A receptor according to claim 1, wherein said receptor comprises a soluble AcPL polypeptide covalently linked to a soluble IL-1Rrp1 polypeptide.
3. A receptor according to claim 1 wherein said receptor comprises IL-1Rrp1 covalently linked to AcPL *via* a peptide linker.
4. A receptor according to claim 3, wherein said receptor is a recombinant fusion protein of the formula:



wherein  $R_1$  represents a soluble IL-1Rrp1;  $R_2$  represents a soluble AcPL, and L represents a peptide linker.

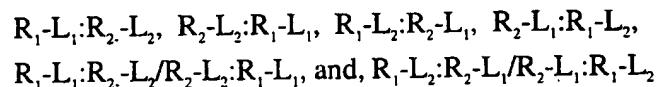
5. A receptor according to claim 4, wherein said soluble IL-1Rrp1 is selected from the group consisting of:

- a) amino acids y-329 of SEQ ID NO:4, wherein y represents an integer between and including 1-20; and
- b) amino acids y'-322 of SEQ ID NO:8, wherein y' represents an integer between and including 1-19;

and, wherein said soluble AcPL is selected from amino acid sequences consisting of:

- a') amino acids x-356 of SEQ ID NO:2, wherein x is an integer between and including 1 and 15; and
- b') amino acids x'-356 of SEQ ID NO:6, wherein x' is an integer between and including 1 and 15.

6. A receptor having a formula selected from the group consisting of:



wherein  $L_1$  is an immunoglobulin heavy chain fragment;  $L_2$  is an immunoglobulin light chain fragment;  $R_1$  is AcPL or an AcPL fragment;  $R_2$  is IL-1Rrp1 or an IL-1Rrp1 fragment; : is a linkage between a heavy chain and light chain antibody region, and / is a linkage between a heavy chain and a heavy chain antibody region.

7. A receptor according to claim 6, wherein said IL-1Rrp1 is a soluble fragment selected from the group of amino acid sequences consisting of:

- a) amino acids y-329 of SEQ ID NO:4, wherein y represents an integer between and including 1-20; and
- b) amino acids y'-322 of SEQ ID NO:8, wherein y' represents an integer between and including 1-19;

and, wherein said AcPL is a soluble fragment selected from amino acid sequences consisting of:

- a') amino acids x-356 of SEQ ID NO:2, wherein x is an integer between and including 1 and 15; and
- b') amino acids x'-356 of SEQ ID NO:6, wherein x' is an integer between and including 1 and 15.



8. An isolated DNA sequence encoding the receptor of claim 7.
9. A recombinant expression vector comprising the DNA sequence of claim 8.
10. A process for preparing a receptor according to claim 6, comprising culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes said fusion protein under conditions that promote expression of said fusion protein, and recovering said fusion protein.
11. A receptor comprising a first fusion polypeptide that comprises an antibody light chain polypeptide attached to the C-terminus of a soluble IL-1Rrp1 or of a soluble AcPL, and a second fusion polypeptide that comprises an antibody heavy chain polypeptide attached to the C-terminus of a soluble AcPL or of a soluble IL-1Rrp1, wherein said first fusion polypeptide is linked to said second fusion polypeptide via disulfide bonds between the heavy chain and light chain polypeptides.
12. A process for preparing a receptor according to claim 11, comprising culturing a host cell co-transfected with a first expression vector encoding said first fusion polypeptide and with a second expression vector encoding said second fusion polypeptide under conditions that promote expression of said first and second fusion polypeptides, and recovering said receptor.
13. A receptor according to claim 11, wherein said IL-1Rrp1 is a soluble fragment selected from the group of amino acid sequences consisting of:
  - a) amino acids y-329 of SEQ ID NO:4, wherein y represents an integer between and including 1-20; and
  - b) amino acids y'-322 of SEQ ID NO:8, wherein y' represents an integer between and including 1-19;and, wherein said AcPL is a soluble fragment selected from amino acid sequences consisting of:
  - a') amino acids x-356 of SEQ ID NO:2, wherein x is an integer between and including 1 and 15; and
  - b') amino acids x'-356 of SEQ ID NO:6, wherein x' is an integer between and including 1 and 15

14. A composition comprising a receptor of claim 2 and a suitable diluent or carrier.
15. A composition comprising a receptor of claim 11.
16. A method for inhibiting the effects of IL-18 comprising administering the receptor of claim 2 to a mammal.

## SEQUENCE LISTING

&lt;110&gt; Immunex Corporation, Sims, John E., Born, Teresa L.

&lt;120&gt; IL-18 Receptors

&lt;130&gt; 2638-WO

&lt;140&gt; --to be assigned--

&lt;141&gt; 1999-01-22

&lt;160&gt; 8

&lt;170&gt; PatentIn Ver. 2.0

&lt;210&gt; 1

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&lt;212&gt; DNA

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Lys Lys Ala Leu Arg Val Leu Pro Thr Val Thr Trp Arg Gly Leu Lys	
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Ser Val Pro Pro Asn Ser Arg Phe Trp Ala Lys Met Arg Tyr His Met	
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Pro Val Lys Asn Ser Gln Gly Phe Thr Trp Asn Gln Leu Arg Ile Thr	
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Ser Arg Ile Phe Gln Trp Lys Gly Leu Ser Arg Thr Glu Thr Thr Gly	
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Arg Ser Ser Gln Pro Lys Glu Trp	
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&lt;211&gt; 599

&lt;212&gt; PRT

&lt;213&gt; Human AcPL

&lt;400&gt; 2

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 Thr Tyr Ser Thr Arg Ser Glu Glu Glu Phe Val Leu Phe Cys Asp Leu  
 35 40 45  
 Pro Glu Pro Gln Lys Ser His Phe Cys His Arg Asn Arg Leu Ser Pro  
 50 55 60  
 Lys Gln Val Pro Glu His Leu Pro Phe Met Gly Ser Asn Asp Leu Ser  
 65 70 75 80  
 Asp Val Gln Trp Tyr Gln Gln Pro Ser Asn Gly Asp Pro Leu Glu Asp  
 85 90 95  
 Ile Arg Lys Ser Tyr Pro His Ile Ile Gln Asp Lys Cys Thr Leu His  
 100 105 110  
 Phe Leu Thr Pro Gly Val Asn Asn Ser Gly Ser Tyr Ile Cys Arg Pro  
 115 120 125  
 Lys Met Ile Lys Ser Pro Tyr Asp Val Ala Cys Cys Val Lys Met Ile  
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 145 150 155 160  
 His Lys Gln Asp Leu Leu Leu Gly Ser Thr Gly Ser Ile Ser Cys Pro  
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 Ser Leu Ser Cys Gln Ser Asp Ala Gln Ser Pro Ala Val Thr Trp Tyr  
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 Lys Asn Gly Lys Leu Leu Ser Val Glu Arg Ser Asn Arg Ile Val Val  
 195 200 205  
 Asp Glu Val Tyr Asp Tyr His Gln Gly Thr Tyr Val Cys Asp Tyr Thr  
 210 215 220  
 Gln Ser Asp Thr Val Ser Ser Trp Thr Val Arg Ala Val Val Gln Val  
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 Arg Thr Ile Val Gly Asp Thr Lys Leu Lys Pro Asp Ile Leu Asp Pro  
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 260 265 270

Cys Lys Ala Arg Phe Gly Phe Glu Arg Val Phe Asn Pro Val Ile Lys  
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 Cys Phe Val Gln Asn Ser Ile Gly Asn Thr Thr Gln Ser Val Gln Leu  
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 Gly Thr Leu Val Ala Val Leu Ala Ala Ser Ala Leu Leu Tyr Arg His  
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 Thr Leu Gly Asp Lys Lys Asp Phe Asp Ala Phe Val Ser Tyr Ala Lys  
 405 410 415  
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 420 425 430  
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 450 455 460  
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 465 470 475 480  
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 485 490 495  
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 Lys Phe Cys Tyr Phe Gln Glu Pro Glu Ser Leu Pro His Leu Val Lys  
 515 520 525  
 Lys Ala Leu Arg Val Leu Pro Thr Val Thr Trp Arg Gly Leu Lys Ser  
 530 535 540  
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 545 550 555 560  
 Val Lys Asn Ser Gln Gly Phe Thr Trp Asn Gln Leu Arg Ile Thr Ser  
 565 570 575  
 Arg Ile Phe Gln Trp Lys Gly Leu Ser Arg Thr Glu Thr Thr Gly Arg  
 580 585 590  
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&lt;210&gt; 3

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Human IL-1Rrp1

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)...(1626)

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Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu	
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Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu	
35 40 45	
att gaa aca acc acc aaa agc tgg tac aaa agc agt gga tca cag gaa	192
Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu	
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cat gtg gag ctg aac cca agg agt tcc tcg aga att gct ttg cat gat	240
His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp	
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tgt gtt ttg gag ttt tgg cca gtt gag ttg aat gac aca gga tct tac	288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr	
85 90 95	
ttt ttc caa atg aaa aat tat act cag aaa tgg aaa tta aat gtc atc	336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile	
100 105 110	
aga aga aat aaa cac agc tgt ttc act gaa aga caa gta act agt aaa	384
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys	
115 120 125	
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Ile Val Glu Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr	
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tat caa aca ctg gtc aac agc aca tca ttg tat aag aac tgt aaa aag	480
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys	
145 150 155 160	
cta cta ctg gag aac aat aaa aac cca acg ata aag aag aac gcc gag	528
Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu	
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ttt gaa gat cag ggg tat tac tcc tgc gtg cat ttc ctt cat cat aat	576
Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn	
180 185 190	
gga aaa cta ttt aat atc acc aaa acc ttc aat ata aca ata gtg gaa	624
Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu	
195 200 205	
gat cgc agt aat ata gtt ccg gtt ctt ctt gga cca aag ctt aac cat	672
Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His	
210 215 220	
gtt gca gtg gaa tta gga aaa aac gta agg ctc aac tgc tct gct ttg	720
Val Ala Val Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu	
225 230 235 240	



ctg aat gaa gag gat gta att tat tgg atg ttt ggg gaa gaa aat gga 768 Leu Asn Glu Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly 245 250 255
tcg gat cct aat ata cat gaa gag aaa gaa atg aga att atg act cca 816 Ser Asp Pro Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro 260 265 270
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gaa agc aat cta aat gtt tta tat aat tgc act gtg gcc agc acg gga 912 Glu Ser Asn Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly 290 295 300
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gat atc cca ggc cac gtc ttc aca aga gga atg atc ata gct gtt ttg 1008 Asp Ile Pro Gly His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu 325 330 335
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gtt gac ttg gtt cta ttt tat aga cat tta acg aga aga gat gaa aca 1104 Val Asp Leu Val Leu Phe Tyr Arg His Leu Thr Arg Arg Asp Glu Thr 355 360 365
tta aca gat gga aaa aca tat gat gct ttt gtg tct tac cta aaa gaa 1152 Leu Thr Asp Gly Lys Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu 370 375 380
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ccc agg gtg ttg gag aaa cat ttt ggg tat aag tta tgc ata ttt gaa 1248 Pro Arg Val Leu Glu Lys His Phe Gly Tyr Lys Leu Cys Ile Phe Glu 405 410 415
agg gat gta gtg cct gga gga gct gtt gtt gat gaa atc cac tca ctg 1296 Arg Asp Val Val Pro Gly Gly Ala Val Val Asp Glu Ile His Ser Leu 420 425 430
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tct aat gag gtc agg tat gaa ctt gaa agt gga ctc cat gaa gca ttg 1392 Ser Asn Glu Val Arg Tyr Glu Leu Glu Ser Gly Leu His Glu Ala Leu 450 455 460
gtg gaa aga aaa att aaa ata atc tta att gaa ttt aca cct gtt act 1440 Val Glu Arg Lys Ile Lys Ile Ile Leu Ile Glu Phe Thr Pro Val Thr 465 470 475 480
gac ttc aca ttc ttg ccc caa tca cta aag ctt ttg aaa tct cac aga 1488 Asp Phe Thr Phe Leu Pro Gln Ser Leu Lys Leu Leu Lys Ser His Arg 485 490 495
gtt ctg aag tgg aag gcc gat aaa tct ctt tct tat aac tca agg ttc 1536 Val Leu Lys Trp Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe 500 505 510

tgg aag aac ctt ctt tac tta atg cct gca aaa aca gtc aag cca ggt 1584  
 Trp Lys Asn Leu Leu Tyr Leu Met Pro Ala Lys Thr Val Lys Pro Gly  
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aga gac gaa ccg gaa gtc ttg cct gtt ctt tcc gag tct taa 1626  
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 Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu  
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 Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu  
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 His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp  
   65                                  70                                  75                                  80  
 Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr  
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 Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile  
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 Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys  
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 Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys  
  145                                  150                                  155                                  160  
 Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu  
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 Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn  
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 Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu  
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 Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His  
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 Val Ala Val Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu  
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 Leu Asn Glu Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly  
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 Ser Asp Pro Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro  
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 Glu Gly Lys Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly  
           275                                  280                                  285

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 290 295 300  
 Gly Thr Asp Thr Lys Ser Phe Ile Leu Val Arg Lys Ala Asp Met Ala  
 305 310 315 320  
 Asp Ile Pro Gly His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu  
 325 330 335  
 Ile Leu Val Ala Val Val Cys Leu Val Thr Val Cys Val Ile Tyr Arg  
 340 345 350  
 Val Asp Leu Val Leu Phe Tyr Arg His Leu Thr Arg Arg Asp Glu Thr  
 355 360 365  
 Leu Thr Asp Gly Lys Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu  
 370 375 380  
 Cys Arg Pro Glu Asn Gly Glu Glu His Thr Phe Ala Val Glu Ile Leu  
 385 390 395 400  
 Pro Arg Val Leu Glu Lys His Phe Gly Tyr Lys Leu Cys Ile Phe Glu  
 405 410 415  
 Arg Asp Val Val Pro Gly Gly Ala Val Val Asp Glu Ile His Ser Leu  
 420 425 430  
 Ile Glu Lys Ser Arg Arg Leu Ile Ile Val Leu Ser Lys Ser Tyr Met  
 435 440 445  
 Ser Asn Glu Val Arg Tyr Glu Leu Glu Ser Gly Leu His Glu Ala Leu  
 450 455 460  
 Val Glu Arg Lys Ile Lys Ile Ile Leu Ile Glu Phe Thr Pro Val Thr  
 465 470 475 480  
 Asp Phe Thr Phe Leu Pro Gln Ser Leu Lys Leu Leu Lys Ser His Arg  
 485 490 495  
 Val Leu Lys Trp Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe  
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      Met Leu Cys Leu Gly Trp Val Phe Leu Trp Phe Val Ala Gly
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Glu Lys Thr Thr Gly Phe Asn His Ser Ala Cys Ala Thr Lys Lys Leu
15      20      25      30
ctg tgg aca tat tct gca agg ggt gca gag aat ttt gtc cta ttt tgt 565
Leu Trp Thr Tyr Ser Ala Arg Gly Ala Glu Asn Phe Val Leu Phe Cys
35      40      45
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Asp Leu Gln Glu Leu Gln Glu Gln Lys Phe Ser His Ala Ser Gln Leu
50      55      60
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Ser Pro Thr Gln Ser Pro Ala His Lys Pro Cys Ser Gly Ser Gln Lys
65      70      75
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Asp Leu Ser Asp Val Gln Trp Tyr Met Gln Pro Arg Ser Gly Ser Pro
80      85      90
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Leu Glu Glu Ile Ser Arg Asn Ser Pro His Met Gln Ser Glu Gly Met
95      100      105      110
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Leu His Ile Leu Ala Pro Gln Thr Asn Ser Ile Trp Ser Tyr Ile Cys
115      120      125
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Arg Pro Arg Ile Arg Ser Pro Gln Asp Met Ala Cys Cys Ile Lys Thr
130      135      140
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Val Leu Glu Val Lys Pro Gln Arg Asn Val Ser Cys Gly Asn Thr Ala
145      150      155
caa gat gaa caa gtc cta ctt ctt ggc agt act ggc tcc att cat tgt 949
Gln Asp Glu Gln Val Leu Leu Leu Gly Ser Thr Gly Ser Ile His Cys
160      165      170
ccc agt ctc agc tgc caa agt gat gta cag agt cca gag atg acc tgg 997
Pro Ser Leu Ser Cys Gln Ser Asp Val Gln Ser Pro Glu Met Thr Trp
175      180      185      190
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Tyr Lys Asp Gly Arg Leu Leu Pro Glu His Lys Lys Asn Pro Ile Glu
195      200      205
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Met Ala Asp Ile Tyr Val Phe Asn Gln Gly Leu Tyr Val Cys Asp Tyr
210      215      220
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Thr Gln Ser Asp Asn Val Ser Ser Trp Thr Val Arg Ala Val Val Lys
225      230      235

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Glu Thr Leu Gly Asp Lys Lys Glu Phe Asp Ala Phe Val Ser Tyr Ser	
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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/01419

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/62 C07K14/715 C07K19/00 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PARNET P. ET AL.: "IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1receptor and its homologues T1/ST2 and IL-1R AcP." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 8, 23 February 1996, pages 3967-3970, XP002091852 cited in the application see the whole document ---	1-16
A	S. A. GREENFEDER ET AL.: "Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 23, 9 June 1995, pages 13757-13765, XP002007499 see the whole document ---	1-16
	--- -/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 May 1999

Date of mailing of the international search report

11/06/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx: 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Mandl, B

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/01419

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TORIGOE K. ET AL.: "Purification and characterization of the human interleukin - 18 receptor." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 41, 10 October 1997, pages 25737-25742, XP002104173 see the whole document ----	1-16
A	US 5 668 256 A (VAN DER HEYDEN JOSE ET AL) 16 September 1997 see the whole document ----	1-16
A	EP 0 759 466 A (HOFFMANN LA ROCHE) 26 February 1997 see page 3, line 9 - line 40 ----	1,6-13, 15
A	WO 96 13593 A (PROCEPT INC) 9 May 1996  see abstract ----	1-5,14, 16
T	T. L. BORN ET AL.: "Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signalling" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 45, 6 November 1998, pages 29445-29450, XP002091850 see the whole document -----	1-16

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/01419

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 16  
is directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/01419

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5668256 A	16-09-1997	AU 657788 B	23-03-1995
		AU 2357192 A	25-03-1993
		CA 2078384 A	19-03-1993
		EP 0533006 A	24-03-1993
		JP 5268967 A	19-10-1993
		NZ 244281 A	27-04-1995
		US 5712121 A	27-01-1998
		US 5455337 A	03-10-1995
		ZA 9206962 A	13-04-1993
EP 0759466 A	26-02-1997	JP 9132598 A	20-05-1997
WO 9613593 A	09-05-1996	NONE	